Lawrence Berkeley National Laboratory
Recent Work

Title
IDENTIFICATION OF RIBULOSE PHOSPHATES IN C14O2 PHOTOSYNTHESIS PRODUCTS

Permalink
https://escholarship.org/uc/item/65f6t3hp

Author
Benson, A.A.

Publication Date
1951-05-15
TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks.
For a personal retention copy, call Tech. Info. Division, Ext. 5545
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
IDENTIFICATION OF RIBULOSE PHOSPHATES IN $^{14}CO_2$

PHOTOSYNTHESIS PRODUCTS

A. A. Benson

May 15, 1951

Berkeley, California
IDENTIFICATION OF RIBULOSE PHOSPHATES IN $^{14}$O$_2$

PHOTOSYNTHESIS PRODUCTS (*)

A. A. Benson

Radiation Laboratory

University of California, Berkeley

The intermediates involved in carbon dioxide fixation by plants are largely phosphorylated hydroxy acids and sugars. A compound observed during the first few seconds of $^{14}$O$_2$ photosynthesis in all the plants investigated in this laboratory has now been identified as ribulose (adenose) diphosphate.

The diphosphate ester occupies a paper chromatographic position near that of fructose and glucose-1,6-diphosphates and 2,3-diphosphoglyceric acid. A monophosphate ester which gives the same labeled sugar upon phosphatase ("Polidase") hydrolysis occupies a chromatographic position intermediate between triose phosphates and the hexose monophosphates. In young cultures of Scenedesmus the concentration of the diphosphate approaches that of phosphoglycerate.

Independent evidence of the phosphorous content of ribulose diphosphate was obtained from measurements of $^{14}/^{32}$P ration in chromatographically separated compounds derived from Scenedesmus saturated with $^{32}$P (12 hours equilibration in radiophosphate) and $^{14}$P (35 minutes photosynthesis in $^{14}$O$_2$). The measured ratios (samples were counted when the ratios were near unity for optimum accuracy) were all multiplied by an appropriate

(*) This work was sponsored by the United States Atomic Energy Commission.
factor to give 3.0 for phosphoglycerate, 5.1 for glucose monophosphate, 5.8 for fructose plus sedoheptulose monophosphate, and 2.0 for ribulose diphosphate. The calculated value for ribulose diphosphate is 2.5.

The chromatographic position of the radioactive sugar $R_f$(phenol) = 0.60; $R_f$(butanol-propionic acid-water) = 0.27) corresponds exactly to that of ribulose prepared by epimerization of ribose or arabinose in pyridine. No common hexoses or tetrose have such a position.

The radioactive sugar resists bromine but is cleaved by oxygen, particularly under basic conditions such as in diethylamine solutions or on anion exchange resins. Radioactive glycolic, glyceric and a polyhydroxy acid (presumably erythronic$^3$) are obtained upon air oxidation. These products are those expected from ribulose oxidation. The labeled diphosphate was found to be oxidized by air in diethylamine solutions to give phosphoglyceric and phosphoglycolic acids as major products. These were identified by chromatography of the hydrolysis products which were found in the expected positions of glyceric acid and glycolic acid$^4$.

The radioactive sugar was epimerized in pyridine$^5$. Co-chromatography of the resultant mixture with ribose and arabinose showed identity of the two major radioactive products with the added sugars. The radioactive 2,4-dinitrophenylosazone$^7$ of D-arabinose was prepared with a tracer quantity of the labeled ribulose. It was found to have the calculated specific activity and this was undiminished by repeated recrystallizations from methyl cellosolve.

The radioactive sugar was catalytically hydrogenated with Adams catalyst and the product was found to co-chromatograph with added ribitol but not with arabinol.
The foregoing observations lead to the conclusion that the radioactive compounds isolated from plants are ribulose 1,5-diphosphate and monophosphate. An examination of the kinetics of formation of this compound from \(^{14}O_2\) during steady state photosynthesis and a discussion of its importance as a \(\text{CO}_2\) donor in the cycle for regeneration of the \(\text{CO}_2\)-acceptors will be published.

REFERENCES


(4) Phosphoglycolic acid observed as a minor radioactive product of \(^{14}O_2\) photosynthesis has been separated and identified. Oxidation during chromatography may provide one source of this phosphoglycolic acid.


(6) The equilibrium mixture of epimers is known to be largely ribose and arabinose. (P. A. Levene and R. S. Tipson, J. Biol. Chem., 102, 563 (1933.) Small amounts of radioactive ribose observed in isolations of ribulose may indicate that it is also present in the original phosphate esters.

(7) C. Neuberg and E. Strauss, Arch. Biochem., 11, 457 (1946).